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Effect of Artificial Insemination Timing on Conception Rate in Lactating Holstein-Friesian Cows

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ABSTRACT

Successful pregnancy in dairy cows requires accurate timing of artificial insemination (AI). Artificial insemination conducted in the morning, midday, and afternoon exposes cows to different environmental temperatures with high ambient temperatures can potentially reduce the viability of spermatozoa, thus disrupting the fertilization process and increasing the possibility risk of pregnancy failure. The purpose of the present study was to ascertain and determine how the effect of various AI timings (during the morning, midday, and afternoon) affected the pregnancy success of the pregnancies in Holstein-Friesian cows. The purposive sampling was used to select a total of 191 Holstein-Friesian cows based on the following specific criteria; cows showing symptoms of estrus, having healthy reproductive organs, having one parturition at least once, aged 2-6 years old, having body condition score of 2.5-3.5 (on a 1-5 scale). The cows were divided into 3 three treatment groups including T1 cows inseminated from 06.00 am to 10.59 am (n=38 cows), T2 cows inseminated from 11.00 am to 03.59 pm (n=82), and T3 cows inseminated from 04.00 pm to 08.59 pm (n=71). The non-return rate was monitored at 19-22 days post-insemination (NRR-1) and 39-42 days post-insemination (NRR-2) was monitored, while the conception rate (CR) was assessed on day 60 post-insemination. Artificial insemination was performed 8 hours after estrus. NRR-1 values for T1, T2, and T3 were 82%, 80%, and 89%, respectively, The NRR-2 values were 71% in T1, 66% in T2, and 79% in T3. The CR for T1, T2, and T3 were 50%, 48%, and 54%, respectively. Although AI timing did not yield conception rates based on AI timing did not show a significant difference in conception rates, artificial insemination performed in the morning insemination is recommended due to its higher likelihood of successful pregnancy compared to other times.

Keywords: Artificial insemination, Conception rate, Dairy cow, Insemination timing, Lactating dairy cows, Non-return rate

INTRODUCTION

In dairy cows, achieving successful pregnancy requires artificial insemination (AI) at the right time. Accurate timing is crucial since AI conducted too early or too late can decrease the probability of pregnancy. Early AI decreases the chance of fertilization (Fernandez-Novo et al., 2020). Spermatozoa will age, and if ovulation occurs during this period, spermatozoa will not be able to fertilize the egg (Hawk, 1987). Spermatozoa that spend too long in the female reproductive tract will experience decreased motility due to excessive capacitation (Aitken et al., 2015). Capacitation is a physiological change that enables spermatozoa to fertilize the egg, but if this process is prolonged without fertilization, spermatozoa may lose their energy and ability to move actively (Mahdavinezhad et al., 2021). Sperm aging plays a critical role in reducing fertility in mammals, as it leads to alterations in membrane integrity and an increase in lipid peroxidation, ultimately decreasing the proportion of viable sperm capable of successful fertilization (Am-in et al., 2011; Donnellan et al., 2022). On the contrary, late AI can lead to the aging of the egg and disrupt the fertilization process (Hunter and Greve, 1997).

According to Roelofs et al. (2006), AI conducted 12-24 hours before ovulation has a high success rate. However, the absence of a definite sign of ovulation is an obstacle for small-scale farmers in determining the optimal AI time. Therefore, AI based on the onset of estrus is an option that can be used by farmers in deciding on an appropriate AI time. The optimal AI time is 8 hours after the onset of estrus (Marques et al., 2024). Therefore, AI conducted at 8 hours after estrus could lead to the possibility of AI in the morning, midday, and afternoon. Artificial insemination conducted during the midday is potentially detrimental to pregnancy success because daytime temperatures are higher than morning and afternoon temperatures (Hamid et al., 2018; Szenci et al., 2018). Elevated temperatures negatively impact sperm quality, potentially reducing pregnancy success (Hansen et al., 2001).

Properly timed AI is essential to maximize the probability of pregnancy on the first insemination attempt. Artificial insemination failure leads to various disadvantages due to longer feed costs, extended time required for conception, and a decrease in the number of offspring that can be born during the lifetime (Tadesse et al., 2022). Therefore, the objective of this study was to investigate whether the morning, midday, and afternoon AI times influence the success of conceptions among Holstein-Friesian cows.

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MATERIALS AND METHODS

Ethical approval

Ethical approval was given by the ethics committee of the Institute of Biosciences, Universitas Brawijaya, Malang, East Java, Indonesia, in compliance with ethical guidelines regarding responsible behavior in the use of Holstein-Friesian Friesian Holstein cows in experimental animal research (Ethical clearance number: 47/EC/KEPK/02/2024).

Animals and treatment groups

The cows used in this study consisted of 191 Friesian Holstein-Friesian cows owned by members of Sinau Andandani Ekonomi (SAE) Cooperative Pujon, a private dairy farm cooperative. In this study, the cows were divided into 3 three treatment groups: Treatment 1 (T1) with 38 cows inseminated from 06.00 am to 10.59 am, treatment 2 (T2) with 82 cows were inseminated from 11.00 am to 03.59 pm, and treatment 3 (T3) with 71 cows were inseminated from 04.00 pm to 08.59 pm. The difference in the number of cows per treatment is due to variations in the availability of cows in each period. The cows included in this study met the following criteria first, they had given birth at least once. Cows that have given birth indicate that the cow had normal reproductive organs capable of pregnancy and giving birth, as evidenced by the cow being able to get pregnant and give birth normally (Diskin, 2014). Second, cows had normal reproductive organs, as evidenced by rectal palpation before artificial insemination (AI). Third, cows were between 2 and 6 years old. According to the report of Susilawati (2014) cows older than six years tend to exhibit lower fertility rates compared to their younger counterpart. Fourth, cows had a Body Condition Score (BCS) of 2.5 to 3.25 (on a 1-5 scale). According to Yamada et al. (2003), BCS of 2.75-3.25 is associated with better AI outcomes. Fifth, the cows exhibited clear signs of estrus.

Artificial insemination

Artificial insemination was carried out by experienced, nationally certified local inseminators who are experienced and nationally certified. To minimize stress during the AI process, cows were handled gently using low-stress handling techniques, ensuring that no force or rough movements were applied. The environment was kept calm and quiet to create a stress-free atmosphere. Furthermore, cows were only inseminated when they showed clear signs of estrus, and the process was conducted swiftly to avoid prolonged handling, ensuring that the cows remained comfortable throughout the process. Artificial insemination was conducted in the 8th hour after the first appearance of estrus. The semen was deposited at the corpus uteri during AI. The frozen semen used in this study was supplied by the Singosari Artificial Insemination Center, and its quality has been previously documented by Yekti et al. (2023). Farmers reporting their cows exhibiting estrous behavior prompted the use of artificial insemination. Insemination was performed eight hours after the onset of estrus signals. Eight hours following the onset of estrus signals, the inseminator inseminated the cows. NNR-1 monitoring was carried out on days 19-22 following AI. Days 39-42 after AI, observation was followed by NRR-2 observation if the cow did not show estrus. Cows that did not show signs of estrus during NRR-1 and NRR-2 observations were confirmed pregnant using the rectal palpation method on day 60 after AI. Rectal palpation, an old long-used, and cost-effective method for diagnosis of pregnancy in cattle, involves manual examination of the uterus to detect the presence of a fetus. Although it provides rapid results, it requires a skilled practitioner and carries the risk of fetal damage (Jaśkowski et al., 2019).

Non-return rate

Non-return rate 1 (NRR-1) was a metric used to evaluate the proportion of cows displaying signs of estrus during the first estrous cycle, specifically between days 19 and 22 post-AI. Non-return rate 2 (NRR-2) measured the percentage of cows that did not exhibit estrus during the second cycle, which occurred between days 39 and 42 after AI. According to Syah et al. (2024), NRR-1 and NRR-2 values were calculated using the following formulas.

NRR1 = (Total inseminated cows – total cows showing signs of estrus on days 19 to 22 / Total inseminated cows) $\times$100

NRR2 = (Total inseminated cows – total cows showing signs of estrus on days 39 to 42 / Total inseminated cows) $\times$100

Cows that did not show any signs of estrus during the NRR observations were assumed to be pregnant and were subsequently examined followed by a pregnancy examination using the rectal palpation method on day 60.

Conception rate

The conception rate (CR) represents the proportion of cows that became pregnant after the first insemination (Souames and Berrama, 2020). This parameter was used to assess the success of pregnancy after cows were considered pregnant based on NRR-1 and NRR-2 observations. Cows not showing signs of estrus at either observation time were subsequently checked for pregnancy using the rectal palpation method, performed on day 60 after AI. According to

Jainudeen and Hafez (2000), CR was calculated as follows: the number of pregnant cows from the first insemination is divided by the total number of cows used in the study multiplied by 100.

CR (%) = (Total cows pregnant at first insemination / Total inseminated cows) $\times 100$

Statistical analysis

Data were tested statistically using R Studio version 4.3.3. This study used a chi-square test to compare the pregnancy success of the three treatments. Statistical significance was expressed at $p \leq 0.05$.

RESULTS AND DISCUSSION

The effect of different AI timings on NRR-1 and NRR-2 is shown in Table 1. No significant differences were found in NRR-1 and NRR-2 values between AI timings ($p > 0.05$). Although there was no significant difference, T2 showed the lowest NRR-1 and NRR-2 values, which were 80% and 66%, respectively. In the present study, a decrease in NRR-1 to NRR-2 values was observed across all treatments, with the largest drop in T2 (14%), indicating that the cows in T2 showed the most estrus signs over the two estrus cycles. In contrast, decreases in NRR-1 to NRR-2 in T1 and T3 were 11% and 10%, respectively.

The NRR-1 values for T1, T2, and T3 were 82%, 80%, and 89%, respectively. An imperfect NRR-1 value means that some cows exhibited signs of estrus in the first estrus cycle after AI. Furthermore, this is explained by the failure of fertilization between spermatozoa and ovum. Moreover, the failure of fertilization might arise from poor semen quality so spermatozoa penetration is not optimal. Alternatively, cows might experience stress, which might lead to reduced quality of the ovum (Walsh et al., 2011). For NRR-2, values were 71%, 66%, and 79%, respectively. There was a decrease in all treatments in the NRR-2 value, attributable to early embryonic death or silent heat (Syah et al., 2024). Early embryonic death can occur if the cow experiences a deficiency of progesterone hormone (Smith et al., 2022), as this hormone functions to maintain pregnancy, and insufficient levels can increase early embryonic mortality (Baruselli et al., 2022). Silent heat, where estrus signs are minimal or absent despite ovulation, can also affect NRR-2 (Sammad et al., 2020). The reduced NRR1 and NRR2 values observed in T2 could be attributed to elevated ambient temperatures. Temperatures in AI at midday (T2) can reach up to 29ºC (Figure 1). High ambient temperatures increase the potential for cows to suffer from heat stress (Herbut et al., 2021). Heat stress is a condition in which certain mechanisms are triggered to maintain the body temperature of cattle when exposed to uncomfortably high temperatures (Dash et al., 2016). Heat stress conditions cause a decrease in the quality of spermatozoa in the female reproductive organs during penetration into the fallopian tubes. Spermatozoa exposed to high temperatures in the female reproductive tract can reduce spermatozoa viability, therefore disrupting fertilization and embryo development (Hansen et al., 2001). Furthermore, while high ambient temperatures may induce stress in cattle, studies have shown that their direct impact on ovulation is less pronounced compared to other reproductive processes (Roth, 2020). Nonetheless, heat stress significantly affects fertility rates and increases early embryonic death risks (Wolfenson and Roth, 2019).

The effects of different AI timings on CR are presented in Table 1. In this study, AI timing did not show a statistically significant difference in pregnancy success ($p > 0.05$). Although the difference between treatments indicated no significant difference, T2 showed the lowest pregnancy success rate, while T3 showed the highest CR value. Furthermore, there was a decrease in the percentage of NRR-2 to CR in all treatment groups. While no significant differences were observed, T2 had the lowest NRR-1, NRR-2, and CR values, indicating that insemination from 11:00 am to 3:59 pm may be associated with higher ambient temperatures compared to the other timings. Research on the effects of AI timing on conception rates in lactating Holstein-Friesian cows remains limited. This lack of research is likely due to several factors, including the historical focus on other reproductive technologies and the assumption that optimal insemination timing has been adequately addressed in standardized protocols. However, the timing of AI can significantly affect conception rates due to physiological variations in the estrous cycles of cows, influenced by factors such as hormonal changes, ambient temperature, and stress levels at different times of the day. Further research into AI timing is essential as understanding the optimal timing of insemination can improve conception rates, especially in lactating cows where reproductive efficiency is critical to maintaining milk production and fertility. Addressing these shortcomings will contribute to refining AI practices and increasing reproductive success in dairy farming operations, especially in areas with variable environmental conditions.

In this study, AI was conducted at the 8th hour after the onset of estrus. Therefore, the time of insemination was matched with the onset of estrus. Cows inseminated at 11:00 am–3:59 pm had a lower conception rate, although there was no significant difference between T1 and T3 ($p > 0.05$). Artificial insemination conducted during the midday (T2) showed no significant difference with AI in the morning (T1) and in the afternoon (T3), so AI can be conducted at any

time as long as the cows are still in the estrus phase. Previous studies have shown that the optimal time is 4–16 hours after estrus (López-Gatius, 2022; Udin et al., 2022).

Artificial insemination conducted more than 16 hours after the onset of estrus tends to have lower conception rates. However, this could be due to the time of ovulation being too close to the time of insemination, as estrus length is generally 24 to 33 hours and spermatozoa need 6-8 hours to capacitate (López-Gatius, 2022; De Rensis et al., 2024). Capacitation, a complicated process occurring in the reproductive organs of females, allows spermatozoa to bind to and penetrate the zona pellucida, facilitating fertilization (Mostek et al., 2021).

These results support previous studies suggesting that the timing between AI and ovulation is crucial for pregnancy success (Lauber et al., 2020). This study also confirms that AI can be done with the "a.m.-p.m. insemination rule", where cows showing estrus symptoms in the morning can be inseminated in the afternoon of the same day (Foote et al., 1979).

Table 1. Non-return rate and conception rate values in Holstein-Friesian cows at Sinau Andandani Ekonomi Cooperative Pujon, Malang, East Java, Indonesia

Variable	T1 (n=38)		T2 (n=82)		T3 (n=71)		P-value
	Not estrus	Pregnant	Not estrus	Pregnant	Not estrus	Pregnant	
NRR1	31 (82%)	-	66 (80%)	-	63 (89%)	-	0.35
NRR2	27 (71%)	-	54 (66%)	-	56 (79%)	-	0.20
CR	-	19 (50%)	-	39 (48%)	-	38 (54%)	0.76

T1: Artificial insemination in the morning (06.00 am - 10.59 am), T2: Artificial insemination in the midday (11.00 am - 03.59 pm), T3: Artificial insemination in the afternoon (04.00 pm - 08.59 pm), NRR1: Non-return rate 1, NRR2: Non-return rate 2, CR: Conception rate

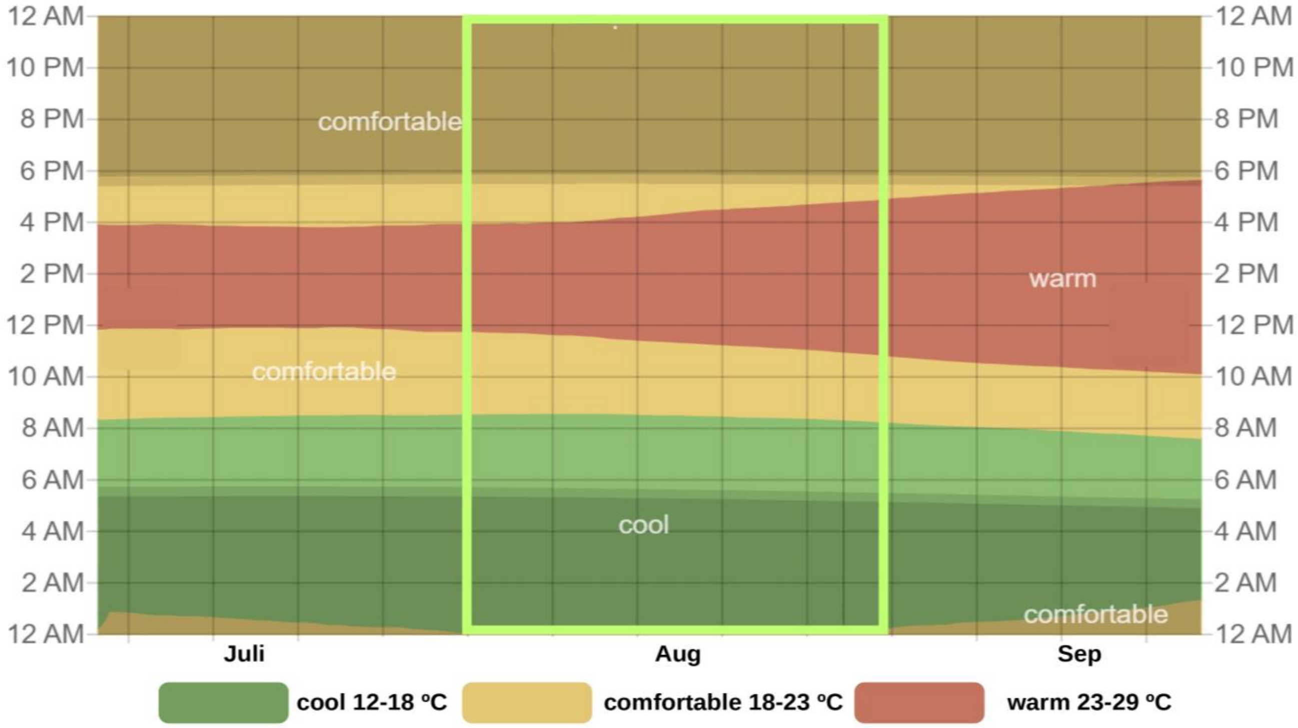

Figure 1. The hourly temperature at Sinau Andandani Ekonomi Cooperative Pujon, Malang, East Java, Indonesia, as recorded by weathers park

CONCLUSION

The timing of AI (morning, midday, and afternoon) in Holstein- Friesian cows did not affect pregnancy success. Although the difference in AI time did not show a significant effect, AI conducted in the midday had the lowest pregnancy success, while AI conducted in the afternoon showed the highest pregnancy success. While AI timing alone (morning, midday, or afternoon) did not significantly affect pregnancy success, further studies are recommended to incorporate additional environmental factors, such as temperature, humidity, and heat stress levels.

DECLARATIONS

Authors' contributions

Habib Asshidiq Syah wrote the manuscript, collected data, analyzed data, and reviewed the final version of the manuscript. Putri Utami reviewed the final version of the manuscript. Aulia Puspita Anugra Yekti, Nurul Isnaini, and Trinil Susilawati designed the study, supervised the study, and reviewed the final version of the manuscript. All authors have approved the final edition of the manuscript manuscript.

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Ethical considerations

All authors have reviewed and confirmed the original content of the article before submission to this journal.

Availability of data and materials

The original contributions of this study are available within the article and its supplementary materials. For further information, please reach out to the corresponding authors.

Competing interests

The authors have not declared any conflict of interest.

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